AGING OF MALE DROSOPHILA MELANOGASTER: HISTOLOGICAL, HISTOCHEMICAL AND ULTRASTRUCTURAL OBSERVATIONS

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CONTENTS

I. INTRODUCTION

As pointed out by Clark and Rockstein (1964), certain characteristics of insects make them especially convenient as experimental animals in aging studies. Drosophila melanogaster is particularly suitable because its life span, even in an optimal environment, is limited to a few months, and large populations can easily be obtained in either homogeneous or genetically pure strains. Furthermore, they can be economically raised under conditions of controlled temperature, relative humidity, standardized diet, etc. Consequently, it is not surprising that Drosophila has become the most widely used arthropod in gerontological studies. In particular, the choice of Drosophila as experimental animal has been very rewarding in the investigation of the effects of the environment on aging. Among the factors investigated have been population density, temperature, starvation, anoxia, hyperoxia and exposure to ionizing radiation. In all these studies longevity has been the only end point. Documentation of histological changes occurring in aging Drosophila is lacking except for observations on the fat body (Norris, cited by Comfort, 1956, p. 98) and various preliminary communications from our laboratory (Miquel et al., 1965/ 1967; Philpott and Miquel, 1967). The aim of the present article is to present additional information on some striking changes occurring in various tissues of aging male Drosophila melanogaster. It is hoped that documenting these changes will stimulate more exhaustive morphological research on the aging process of Drosophila under various environmental conditions. As to the relevance of this work, we realize that great caution should be exerted in extrapolating observations on insects to problems of mammalian aging. Nevertheless, histological studies on Drosophila, which show few mitoses in the adult stage, might contribute to

a better understanding of time-associated changes in post mitotic mammalian cells such as those in the muscle and nervous tissue.

II. METHODS

Virgin Oregon R wild type male Drosophila melanogaster were used in the present study. A population of 450 flies was housed after eclosion in groups of 10 per vial at 22 ±0.5°C and 45 percent relative humidity. The entire population was collected within an interval of 20 hours, so that the age of the insects was known with a maximum error of ±10 hours. They were fed a standard corn meal-molasses-agar medium enriched with brewer's yeast. Once a week the flies were shaken into vials containing fresh food. The data in Figure 1 are based on the mortality of 150 Drosophila. Simultaneously, another population was raised to provide flies of various ages for histological, histochemical and ultrastructural studies. For this purpose, groups of 30 flies each were sacrificed at the following ages: 2, 6, 15, 19, 30, 50, 70, 84, 96 and 100 days post eclosion. In addition, 250 virgin males, ranging in age from 2 hours to 104 days, were obtained from 5 other populations, the first raised 4 years ago and the last in recent months. For routine histological investigation, the following procedures were followed: under light ether anesthesia, the insects were sectioned into head, thorax and abdomen and fixed in Carnoy's fluid or in methanol-38% formalin (6:4) for 1 day at 4°C, followed by 1 day at room temperature. Head, thorax and abdomen were individually embedded in paraffin and serially sectioned at 5μ. They were stained with hematoxylin — eosin and Feulgen — light green to reveal their histological and cytological features. For investigation of protein changes associated with the aging process, the basic fuchsin-amido black-naphthol yellow (FAN) method was used. This method (Miquel

and Calvo, 1958; Miquel et al., 1968) is based on the affinity of tissue proteins for the acid dye amido black, which is widely used for the staining of proteins on electrophoretic strips and has also been recommended for histological demonstration of basic proteins (Seitelberger et al., 1957) and of mitochondria (Benes, 1960). Glycogen was demonstrated employing the periodic acid-Schiff reaction following treatment with dimedon according to Bulmer (1959). The identity of the polysaccharide deposits was confirmed by extraction of parallel sections with amylase prior to staining. Oil red O and Sudan black B were used for histochemical demonstration of lipids. Under light ether anesthesia, the abdomens were dissected and fixed thereafter in 10% formalin during 24 hours. They were then embedded in gelatin and sectioned using a freezing microtome. The sections were stained, rinsed and mounted in glycerin jelly. Search for age pigment was performed by observation of unstained paraffin sections in a Zeiss fluorescence microscope. The following Zeiss equipment was used: light source - Osram HBO 200 high pressure mercury burner; exciter filter - BG 12; barrier filter - OG 5; condenser - dark field; objective - apochromat 100 X, oil immersion.

For both light and electron microscopic studies, the brain and pieces of abdomen were dissected and fixed by immersion in potassium phosphate buffered cold (0-4°C) 2.5% glutaraldehyde (Osborne, 1968) for 20 minutes. Further details of the technique for electron microscopic observations can be found elsewhere (Philpott et al., 1969). Comparative light microscopic studies were performed on 1μ thick sections stained with methylene blue (Philpott, 1966).

III. NORMAL HISTOLOGY OF THE IMAGO OF DROSOPHILA MELANOGASTER

Since we are primarily concerned with the documentation of aging changes, the present article deals very succintly with the normal histology of Drosophila. The reader interested in a detailed description of the cytological and histological characteristics of the normal adult fruit fly should consult the excellent article: "The internal anatomy and histology of the imago of Drosophila melanogaster" by Albert Miller (1965). Additional information on specific tissues can be found in the articles by Hertweck (1931), Strassburger (1932), Gleichauf (1936), Power (1943) and Butterworth and Bodenstein (1968).

Those not familiar with the basic organization of the insect body may benefit from the following description from the article by Miller:

> "Despite their great diversity in anatomical details, all insects have an internal organization and histological structure that are fundamentally uniform throughout the class. The general body plan of the adult pomace fly, Drosophila melanogaster Meigen, is thus typically insectean in character. The skeletal framework of the body consists of a segmented, chitinous external cuticle secreted by a single layer of epidermal cells. The hardened or sclerotized areas of the body wall and appendages are joined by flexible membranes, which permit movement and, in some places, a certain amount of distension by expansion. Sense organs are situated on the surface of this exoskeleton, and muscles are attached to its inner side. A tubular alimentary canal extends through the central axis of the body from the mouth at the anterior pole to the anus at the posterior pole. Above the alimentary

canal there is a mediodorsal blood vessel through which the blood passes forward to the head, where it is poured into the haemocele, or definitive body cavity, to flow backward and bathe all the organs. The central nervous system consists of a dorsal brain in the head and a medioventral ganglionated nerve cord below the alimentary canal. Respiration is effected through a system of branching tubes that open to the outside through lateral breathing pores and permeate all the tissues of the body. The reproductive organs in both sexes are situated posteriorly and have an external opening below and morphologically anterior to the anus. Masses of fat tissue lie within the haemocele. Excretory and incretory (endocrinal) functions are subserved by special organs closely associated with the digestive and circulatory systems. The predominant cellular elements of the body comprise epithelia, muscle, fat and nerve tissue. In addition, there are blood cells, special secretory elements and sex cells. Except for the adipose tissue, connective tissue of the type found in vertebrates and mesoglial or parenchymatous tissue are entirely absent."

IV. AGING CHANGES

A. Digestive System

Usually the œsophagus, crop, anterior and posterior intestines and rectum (Fig. 2) appeared normal in old* flies. In some senescent Drosophila the digestive cells of the ventricular epithelium were not so basophilic and its "brush" border not so conspicuous as in the young individuals

*We have divided arbitrarily the life span of Drosophila imagoes in three phases: young age: from eclosion to 30 days; middle age: 31 to 69 days and old age: 70 days to maximum life span. In the present article senescent is considered to be synonymous with old.

(Fig. 3A, B). The most striking changes occurred in the cardia, a saccular modification of the ventricular wall which covers the valve formed by the invaginated end of the oesophagus (Fig. 2). The epithelial cells of the cardia, which were very basophilic in the young flies (Fig. 3C), lost their affinity for hematoxylin, gallocyanin and basic fuchsin in old Drosophila (Fig. 3D). Another time related change was an increase in the number and size of the vacuoles observed in the cardia cells.

B. Circulatory System and Associated Tissues

The circulatory system of Drosophila is of the "open" type containing a dorsal vessel, the haemocele or body cavity, accessory pulsatile organs and the haemolymph which surrounds all the internal organs (Fig. 4). Associated with the circulatory system are the oenocytes and adipose tissue (Fig. 5).

1. Haemocele.

The haemocele is the general body cavity containing the blood which fills the narrow gaps between the internal organs. In most old flies, the organs were probably shrunk, with the result that the haemolymph filled space was larger than in young Drosophila (Figs. 6 and 7).

2. Oenocytes.

In the abdomen of young imagoes the oenocytes can be seen as large colorless cells. They are strongly acidophylic and have a dense cytoplasm. Their role is still not elucidated, but it seems probable that they are involved with intermediary metabolism or internal secretion.

The most obvious change occurring in the oenocytes of aging Drosophila is a darkening due to the accumulation of a brown pigment (Fig. 8). When excited with blue-ultraviolet light, the pigment emits an orange

fluorescence. Electron microscopic observation shows that in some instances the pigment granules are surrounded by mitochondrial structures (Fig. 9).

3. Adipose tissue.

This tissue is formed by masses of fat cells and is also named the "fat body." The cells are arranged in thin sheets that fill most of the interstitial space of the body. The cytoplasm of the fat body of a young Drosophila imago is packed with protein, glycogen and fat, showing that this tissue is an important store house. Recent work has demonstrated that the fat body is not a relatively inert organ, but combines some of the characteristics of the mammalian adipose tissue and liver (Shigematsu, 1958; Candy and Kilby, 1959; Wigglesworth, 1961; Orr, 1964).

Our conclusions on the aging changes of the adipose tissue are based on observations performed on the head, thorax, and abdomen. However, special attention was given to the fat body masses situated in the head, between the eyes and the brain (Fig. 6). These masses are easily identifiable and comparisons can be made without difficulty between flies of different ages. Maximum development of the adipose tissue was shown by 5 to 15 day old Drosophila. At later age some shrinkage of the fat body was apparent in most flies (Fig. 6). In hematoxylin and eosin and FAN preparations (Fig. 10) of imagoes 2 to 15 days of age, the fat body cells appeared as prisms showing distinct cellular membranes. The cytoplasm stained homogeneously with amido black, except for a meshwork of strands radiating from the nucleus which showed greater affinity for the dye. Rounded empty spaces, occasionally as large as the cross section of the cells, suggested the presence of abundant fat droplets in the living tissue. At 15 days post eclosion,

the fat body cells had a spongy appearance which was still more manifest at 30 days. This was interpreted as indicating the presence of abundant small globules of fat separated by narrow rims of background cytoplasm. At 30-100 days of age, abundant granules of protein were present in the cytoplasm. The granularity seemed to progress with age, being one of the signs of a gradual disorganization which in some instances was also revealed by a reduction in size of the fat bodies and cellular lysis. Variability of the nuclear size was also observed in the adipose tissue of senescent Drosophila. Some flies 104 days old at the time of their sacrifice showed a completely different appearance of the fat body (Fig. 10E). The cells looked shrunken and clearly separated and the cytoplasm stained intensely with basic fuchsin. The appearance was strikingly similar to that of 67 days old Drosophila which were fasted during 24 hours before sacrifice (Fig. 10F).

Changes observed in the abdominal adipose tissue closely paralleled those occurring in the cephalic fat body with the following exception: surviving larval adipose cells were seen in sections of 2 day old flies stained by the FAN procedure. These cells had large nuclei and proteinaceous globules which stained very intensely with amido black. The larval cells were no longer present in Drosophila 6 days of age or older.

The dimedon-PAS procedure when applied to sections of young Drosophila resulted in a very positive staining of the adipose tissue, because of its glycogen content (Fig. 11). The polysaccharide was very abundant in 2 day old flies, filling uniformly the cytoplasm with the exception of the rounded spaces occupied by the fat droplets. The cellular membranes were occasionally outlined by the lack of staining. At 5 days, the staining was still very intense but the number of empty spaces had increased. At 16 days

the adipose tissue appeared spongy because of a further increase in the number of fat droplets. At 30-54 days the glycogen deposits were finely granular and the empty spaces were abundant and of smaller size than those observed in younger flies. Senescent Drosophila showed shrunken fat bodies, with glycogen concentrated in clumps while some areas were devoid of it (Fig. 11C). A practically complete loss of glycogen was observed in 104 day old flies and also in the fasted insects (Fig. 11D).

Changes in the lipid distribution also seemed to occur with age. Recently emerged imagoes showed very large fat droplets (Fig. 12A) which were no longer apparent in flies 7 days and older (Fig. 12B). The number of lipid droplets seemed to increase from 7 to 30 days and striking changes were observed in senescent individuals. Whereas in 30 day old flies the fat was present in discrete globules (Fig. 12C), it had a tendency to coalesce in old Drosophila (Fig. 12D). The amount of fat ranged widely in old flies, some having abundant deposits, whereas in other individuals, histochemically demonstrable lipids were almost nonexistent.

C. Excretory System

The excretory system is constituted by the Malpighian tubules, two pairs of long channels connected by single stalks to the ventriculus (Fig. 2). The tubules are formed by large epithelial cells enclosing a lumen lined by a "brush border." The waste products that accumulate in the Malpighian tubles are eliminated by way of the intestine.

Some senescent Drosophila showed a moderate sponginess in the cytoplasm of the Malpighian cells (Fig. 13) and granular deposits, which are probably identical to the substances observed by Wigglesworth (cited by

Miller, 1965, p. 435) in old flies and tentatively identified by him as calcium salts.

D. Muscular System

We have focused our attention on the glycogen changes occurring in the thoracic muscles, which are responsible for the movement of the head, legs and wings. Histochemically, demonstrable glycogen reached its maximum in 2-30 day old flies, decreased in older Drosophila and in some 84-100 day old individuals was practically nonexistent (Fig. 14).

E. Nervous System

Our observations are limited to the brain (Fig. 15). This organ contains nerve cells and glial cells and is enclosed by a nucleated neurilemma. The nerve cell bodies or neurocytes are localized in the peripheral region forming the cortex, while their processes constitute the neuropil (Fig. 16).

Degenerative changes are apparent in the "giant" nerve cells, which probably have a neurosecretory function. The changes are very striking in the cells of the anterior middle grove immediately dorsal to the oesophageal canal. These neurons which, due to the basophilia of their cytoplasm, can be very easily demonstrated in sections of young flies (Fig. 16A) are not so conspicuous in middle aged individuals and have lost almost completely the affinity for basic dyes in old Drosophila. Similar degenerative changes have been observed in the giant neurons of the intercerebral furrow (Figs. 16B, C, D) and in those immediately under the terminal expansion of each posterior root of the corpora pedunculata.

The most abundant cellular elements of the brain are the "large" and "small" neurons, which have a nucleus 2-4μ in diameter and very scanty cytoplasm (Fig. 17). That many of these cells may disappear in old Drosophila is suggested by the shrinkage of the cortical areas which is apparent in most 70-100 day old flies (Fig. 6). Pathological changes in the neurons of senescent flies are clearly demonstrated by electron microscopy (Figs. 18 and 19). Other time related alteration was certain "sponginess" of the neuropil first noticeable in approximately 50% of the 70 day old Drosophila and present in almost all 84-100 day old flies.

F. Reproductive System

The organization of the male reproductive system can be seen in Fig. 20. The most obvious time related changes were noticed in the testes, accessory glands and anterior ejaculatory duct. In the testes, there was a decrease in the number of spermatogonia and spermatocytes. Moreover, the spermatozoa, which were neatly arranged in bundles in the young flies, showed signs of disorganization in old Drosophila. Also, in senescent flies the epithelium of the anterior ejaculatory duct showed loss of the nuclei and ballooning of the cytoplasm (Fig. 21). There was also a change in the fluid content of the accessory glands. The coagulated fluid, which was finely granular in young Drosophila, had a coarse appearance in senescent flies (Fig. 21).

V. COMMENT

The interpretation of time associated changes at the tissue level is fraught with difficulties. As pointed out by Strehler (1962), "the products of disease and the sequelae of long continued illness may ... give the

impression of atrophic or other changes which are not necessarily a part of the normal aging process." Nevertheless, most changes described in this article were universally present in old flies of a colony, which although containing virus-like particles in some of their tissues (Philpott et al., 1969), on the basis of its mortality dynamics seemed unusually healthy. The survivorship curves tended to be rectangular in shape, which is the case for homogeneous populations living in an optimum environment, and the maximum recorded longevity was 132 days. Although no certainty can exist until the pathologic processes of Drosophila are better known, we feel tempted to speculate that most changes reported in this article are true time related degenerative changes and not the result of specific diseases.

From the data on structural changes in aging insects, it seems that some of the degenerative processes suffered by Drosophila may also occur in other species. In effect, Norris, cited by Comfort (1956, p. 98) has described a depletion of the adipose tissue which is a common feature of senescence in Drosophila, Ephestia, Carabus and Sitodrepa. Poor appearance of the adipose tissue was also observed by Haydak (1957) in bees older than 25 days. Similar to our observations on Drosophila, the oenocytes of adult bees had greenish-brown granules which increased with age. Indeed, Haydak (1929, cited by Haydak, 1957) observed that the age of bees could best be determined by the color of their oenocytes. With regard to degenerative changes in the brain, our observations agree substantially with the findings of Hodge, cited by Clark and Rockstein (1964, p. 239) and of Rockstein (1950) about cell depletion in the honey bee brain. Neuron loss seems to be an age related structural change common to both insects and higher animals. In Rockstein's opinion (1950), the nerve cell depletion in the brain of the honey bee follows a pattern almost identical to that reported for the human brain.

Further similarities in the aging process of the nervous tissue exist. In humans, brain weight seems to decrease at a uniform rate with age (Appel and Appel, 1942). Also a decrease in brain volume relative to the capacity of the skull has been observed, resulting in a shrunken appearance of the brains of senile individuals. These changes seem to have an exact counterpart in senescent Drosophila. The parallelism would also be present at the microscopic level, since cell shrinkage and cytoplasmic atrophic changes occur during senescence of both human subjects and Drosophila. Further, decreased cytoplasmic basophilia which we observed in the giant nerve cells of Drosophila has also been reported in mouse and human Purkinje cells (Andrew, 1937), rat cerebral cortical cells (Kuhlenbeck, 1954) and nerve cells of the human pallidum (Klatzo, 1954).

In general, differences observed by us between young and old Drosophila correspond well to those which, according to Strehler (1963), exist between young and old human beings, i.e., decrease in the regularity of arrangement of cells as an individual grows older, greater variability of nuclear size and intensity of the staining reaction in aged individuals and more homogeneous appearance of the cells in young individuals than in the old.

Another common characteristic of mammalian and insect aging seems to be the accumulation of age pigment. In our opinion, the granules observed by us in the oenocytes are true age pigment, since they increase continuously with age and are similar in color and size to the mammalian lipofuscin. However, important differences exist. Whereas dark granules have been observed in great amounts in the oenocytes and in lesser concentrations in the fat body and digestive tract, they are not present in the tissues showing the heaviest deposits in mammals (muscle and nervous tissue). Furthermore, the granules

seen in the oenocytes exhibit a dull orange fluorescence instead of the brilliant orange characteristic of the lipofuscin. A mitochondrial origin of the mammalian pigment has been proposed by Hess (1955) and by Duncan et al., (1960). According to Hess, the mitochondria vacuolate and the vacuoles coalesce to form pigment. The most widely accepted opinion, however, is that the lipofuscin granules have a lysosomal origin. In the oenocytes, the pigment does not show any relation to lysosomal structures. On the contrary, in some instances the granules are found in close spatial relationship to mitochondria. Therefore, the age pigment of Drosophila seems more akin to the mitochondrial pigment found by Rudzinska (1961a, b) in the protozoan Tokophrya infusionum than to the typical lipofuscin of the mammalian tissues.

Until the mechanisms involved in Drosophila aging are better known, the relative importance of the various tissues in producing physiological senescence can only be conjectured. It is a reasonable assumption that the degeneration of the fat body must have an essential role in the changes conducive to senescence and death. The fat body synthetizes and stores carbohydrates, fat, and proteins and mobilizes them when required as a source of energy or for repair of the tissues. Besides being a storehouse, it is a regulator of various blood constituents and is involved in detoxification (Kilby, 1965). In connection with our findings of both neurosecretory cell degeneration and fat body involution in senescent Drosophila, the following is of interest. There are neurosecretory cells in the insect brain responsible for the production of a hormone which passes along the axons to the corpora cardiaca for storage in this organ and eventual release into the blood (Scharrer, 1952). Steele (1963) and Bowers and Friedman (1963) have shown that injecting this hormone into the cockroach results in a sharp rise in the blood trehalose and a fall in the glycogen level of the fat body. Moreover, according to

Shigematsu (1958), the insect fact body can synthestize some of the blood proteins. When the neurosecretory cells are destroyed, the blood protein level falls to a half or a third of its normal value. In view of the above observations, it is plausible to speculate that when both the fat body and the neurosecretory cells degenerate in old Drosophila, severe disturbances in intermediary metabolism and homeostasis will ensue. These disturbances might very well cause the insects' death.

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LEGENDS

Figure 1. Survivorship curve of Drosophila melanogaster used in this study.

Figure 2. Digestive system. Top: lateral aspect. Bottom: dorsal aspect. AInt, anterior intestine; An, anus; Br, brain; Car, cardia; Cb", posterior plate of cibarium (sucking pump); Cr, crop; Mal', anterior, Mal" posterior Malpighian tubules; Oe, oesophagus; Ptl, ptilinum; Rect, rectum; SlD, salivary duct; SlGl, salivary gland; ThGng, thoracic ganglion; Vent, ventriculus. (Drawn after Miller, 1965)

Figure 3. Sections of the digestive canal. (A) Ventriculus of a 6 day old imago. (B) Ventriculus of a 84 day old imago, showing a decrease in basophilia of the epithelial cytoplasm. FAN stain (X250). (C) Cardia of a 6 day old imago. (D) Cardia of a 84 day old imago (note loss of basophilia and increased vacuolation). Hematoxylin-eosin (X250).

Figure 4. Circulatory system. AlMsc, alary muscle of heart; Ao, aorta; AoFnl, aortic funnel; CA, corpus allatum; Hmcl, haemocele; Ht_1, first, Ht_4, fourth chamber of heart; Nph, thoracic nephrocytes; Ost_1, Ost_4, ostia; PCl, pericardial cells; SclPO, scutellar pulsatile organ; Sep, longitudinal septum of leg. (Drawn after Miller, 1965)

Figure 5. Distribution of adipose tissue and oenocytes; the deep fat of the abdomen has been omitted. Top: mesal aspect of right half of body, female. Bottom left: ventral aspect of dorsal half of body, male. Bottom right: dorsal aspect of ventral

half of body, male. Dft, deep fat; Oen, oenocytes; PcFt, pericardial fat; PFt, peripheral fat. (Drawn after Miller, 1965)

Figure 6. Vertical transections of the head. (A) 5 days old, showing normal appearance of the cortical layers, neuropil and two giant nerve cells located immediately dorsal to the oesophagus. The fat body situated between the brain and the eye has the structure usually found in young flies. (B) 70 days old. Both the fat body and the brain are shrunken, with a concomitant increase in the haemocele. The cortical layers are reduced in thickness and the neuropil shows moderate "sponginess." FAN stain (X250).

Figure 7. Vertical transection of the abdomen. (A) 15 day old imago. (B) 84 day old imago, showing enlargement of the haemocele. FAN stain (X160).

Figure 8. Oenocytes. (A) 7 day old imago. (B) 84 days old. The oenocytes appear darker due to increased content of age pigment. Sudan black B (X250). (C) 1μ thick section of a 30 day old fly showing moderate amount of pigment granules. (D) 1μ thick section of an 85 day old fly (note the abundant granules). Methylene blue stain (X520).

Figure 9. Electron micrograph of oenocyte of a 76 day old imago showing abundant osmiophilic granules. Virus-like particles are present in the nucleus (X15,000).

Figure 10. Cephalic fat body of Drosophila imagoes showing disorganization induced by aging and by starvation. Age: (A) 2 days; (B) 30 days; (C) 70 days; (D) 91 days; (E) 104 days; (F) 67 days, fasted during the 24 hours preceding sacrifice. FAN stain (X400).

Figure 11. Changes in the glycogen content of the cephalic fat body. Age: (A) 2 days; (B) 30 days, showing granular deposits; (C) 100 days (note the glycogen loss in the upper area); (D) 67 days, fasted during the 24 hours preceding sacrifice (note the decrease in glycogen content). Dimedon-PAS-gallocyanin stain (X250).

Figure 12. Fat distribution in the abdominal adipose tissue. (A) 1 hour old imago, showing very large fat globules. (B) 15 days old (note the decrease in size of the fat droplets). (C) Abundant fat in a 30 day old. (D) Loss of organization of the fat body in an 84 day old. Oil red O stain (X250).

Figure 13. Malpighian tubules, showing some sponginess and deposits (calcium salts?) in the old imagoes. (A) Posterior Malpighian tubule of a 5 day old imago. (B) Posterior tubule of a 100 day old imago. (C) Terminal portion of an anterior tubule of an 84 day old imago. FAN stain (X400).

Figure 14. Loss of the glycogen content of the thoracic muscles. Age: (A) 5 days. (B) 88 days. Dimedon-PAS-gallocyanin (X250).

Figure 15. Dorsal view of ventral half of male, showing the central nervous system. AntNv, antennal nerve; 1 Br, protocerebrum; 2 Br, deutocerebrum; CvCon, cervical connective; E, compound eye; O, ocelli; OPdcl, ocellar pedicel; OpL, optic lobe; SoeGng, suboesophageal ganglion; ThGng, thoracic ganglionic mass.

Figure 16. Effects of aging on the "giant" nerve cells of the brain. (A) Vertical transection of the head of a 7 day imago showing two giant nerve cells in the central area of the brain. (B) Section of the same brain showing a cluster of giant neurons of the intercerebral furrow, under the ocelli. Hematoxylin-eosin (X160). (C) Basophilic giant neurons in the intercerebral furrow of a 5 day imago. (D) Degenerating nerve cells in the same location in an 84 day old imago. Gallocyanin-dimedon-PAS stain (X400).

Figure 17. Electron micrograph of nerve cells from the cortex of a 7 day old imago (X9300).

Figure 18. Electron micrograph of nerve cells from the cortex of a 91 day old imago. Some of these neurons show a drastic reduction of the cytoplasmic area. Conversely other nerve cells are strikingly swollen (X9300).

Figure 19. Abnormal dark bodies originating from mitochondria in the cortex of a 91 day old imago (X21,540).

Figure 20. Male reproductive system. AcGl, accessory gland; A Int, anterior intestine; An, anus; Bej, ejaculatory bulb; Cr, crop; Dej', anterior ejaculatory duct; Dej'', posterior ejaculatory duct; msc, muscle fibers; Rect, rectum; Tes', left testis; Tes'', right testis; TesD, testicular duct; Vd, undistended portion of vas deferens; Vent, ventriculus; Vsm, seminal vesicle. (Drawn after Miller, 1965)

Figure 21. Effects of aging on the accessory glands and anterior ejaculatory duct. (A) 7 day old imago. (B) 84 day old imago, showing ballooning of the epithelial cells of the duct. Note also the coarse granular appearance of the content of the accessory glands. Hematoxylin-eosin (X250).

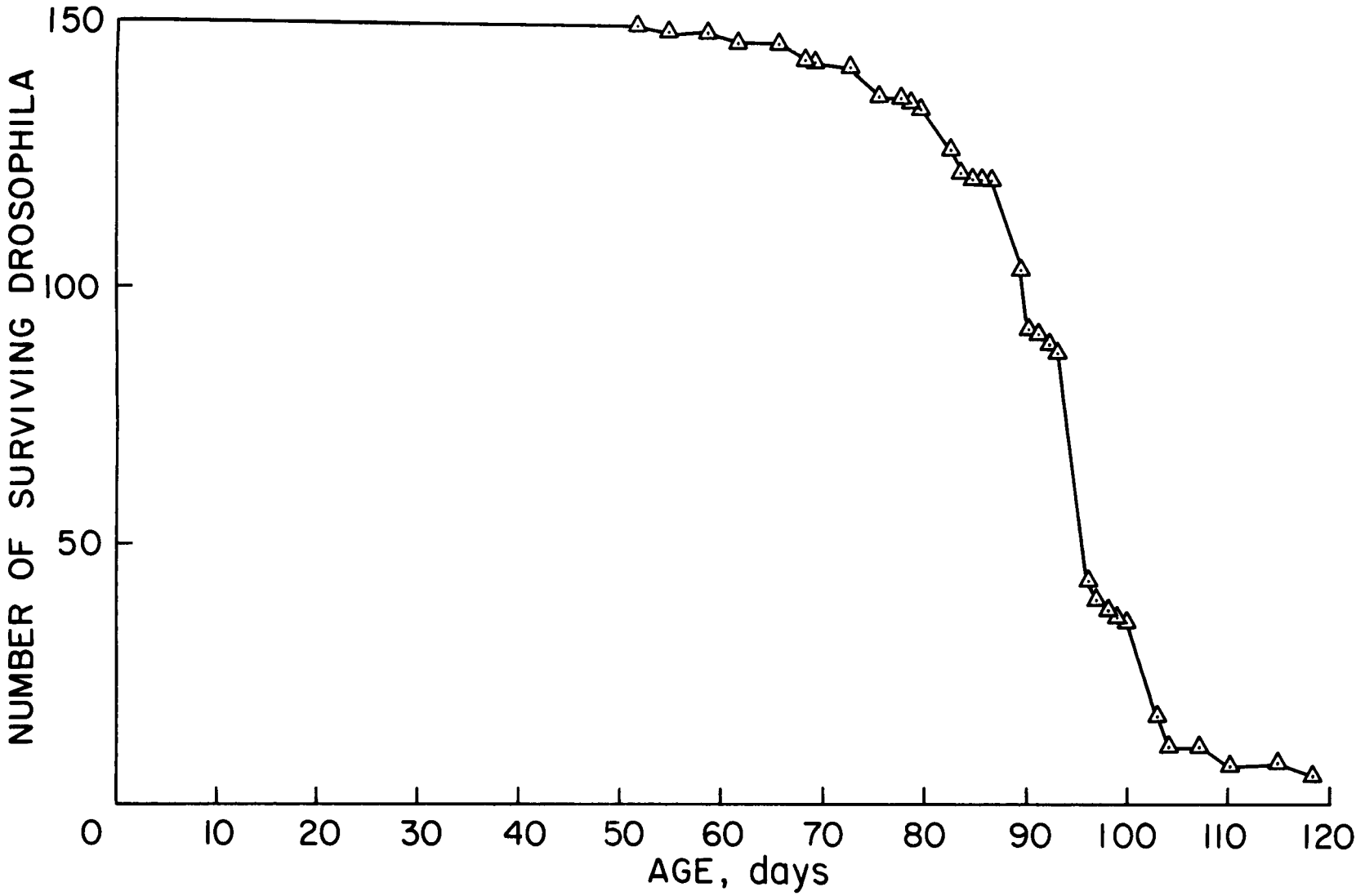

Figure 1.

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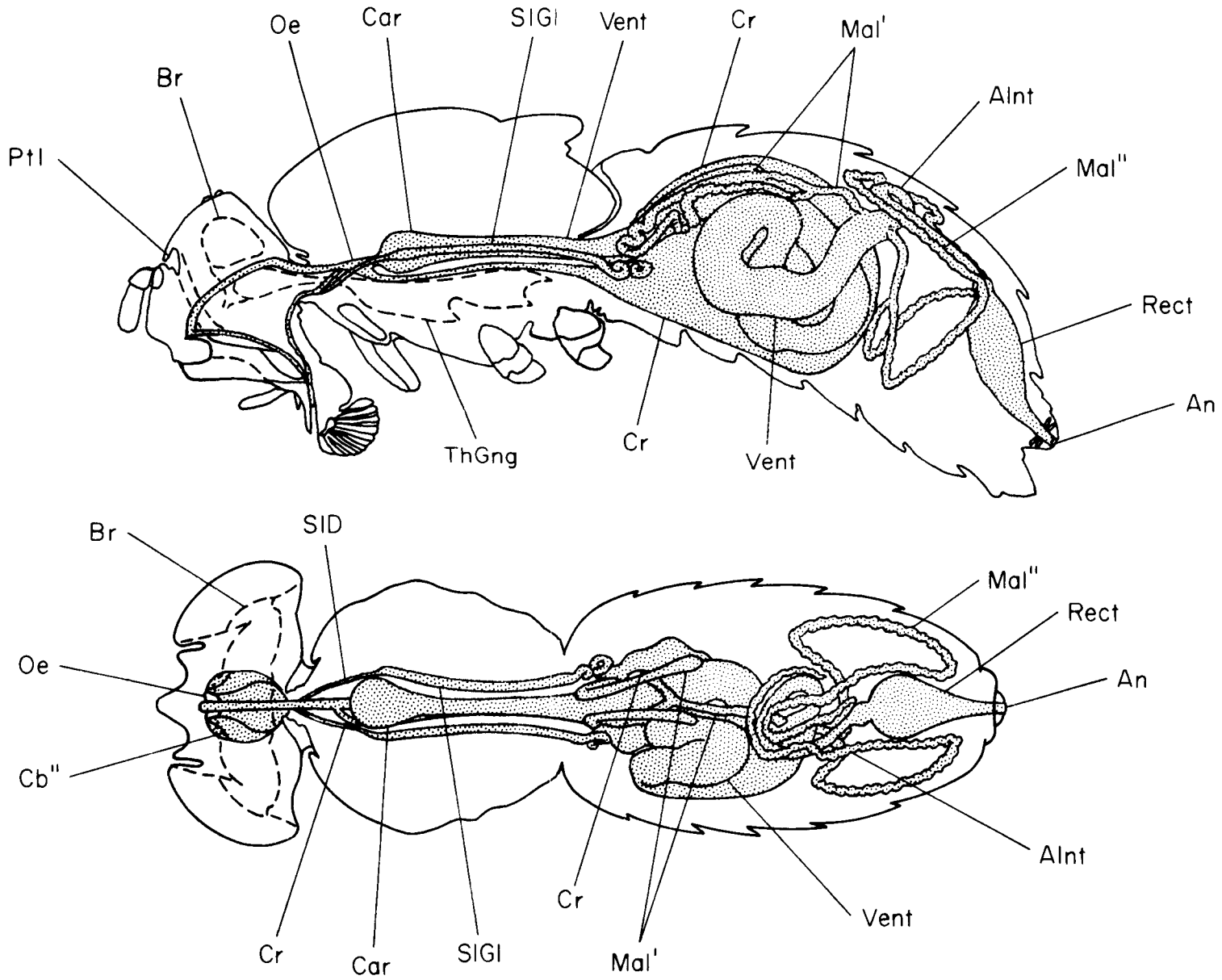

Figure 2.

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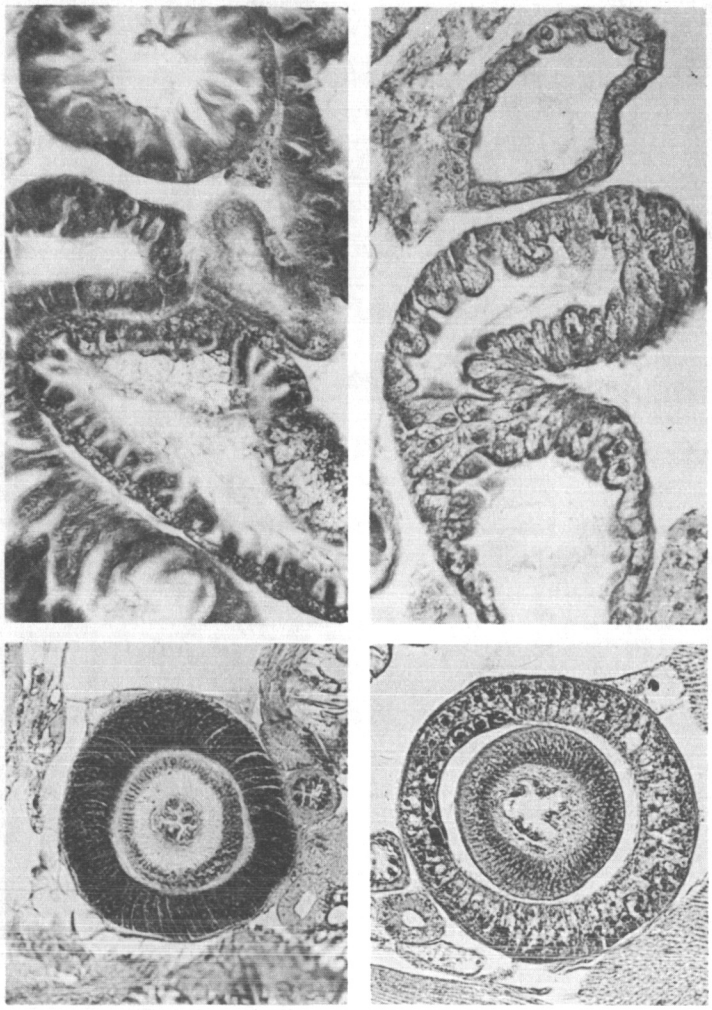

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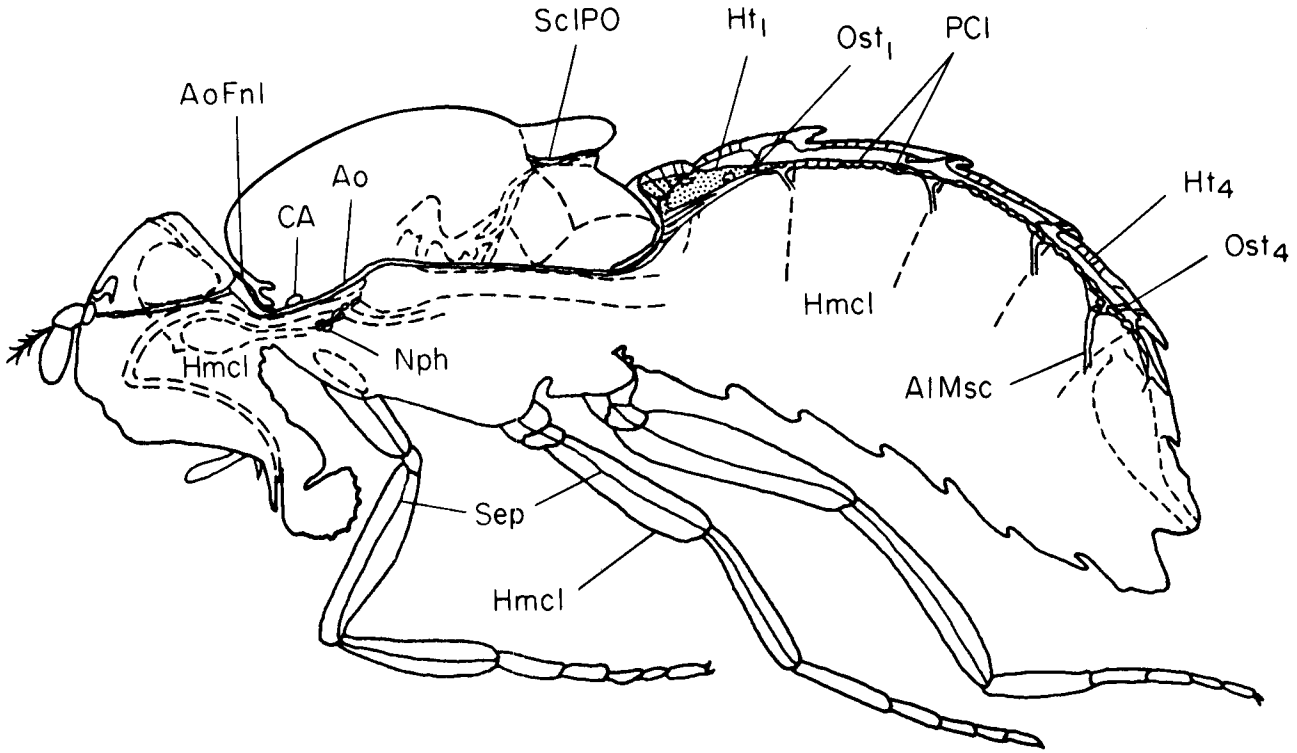

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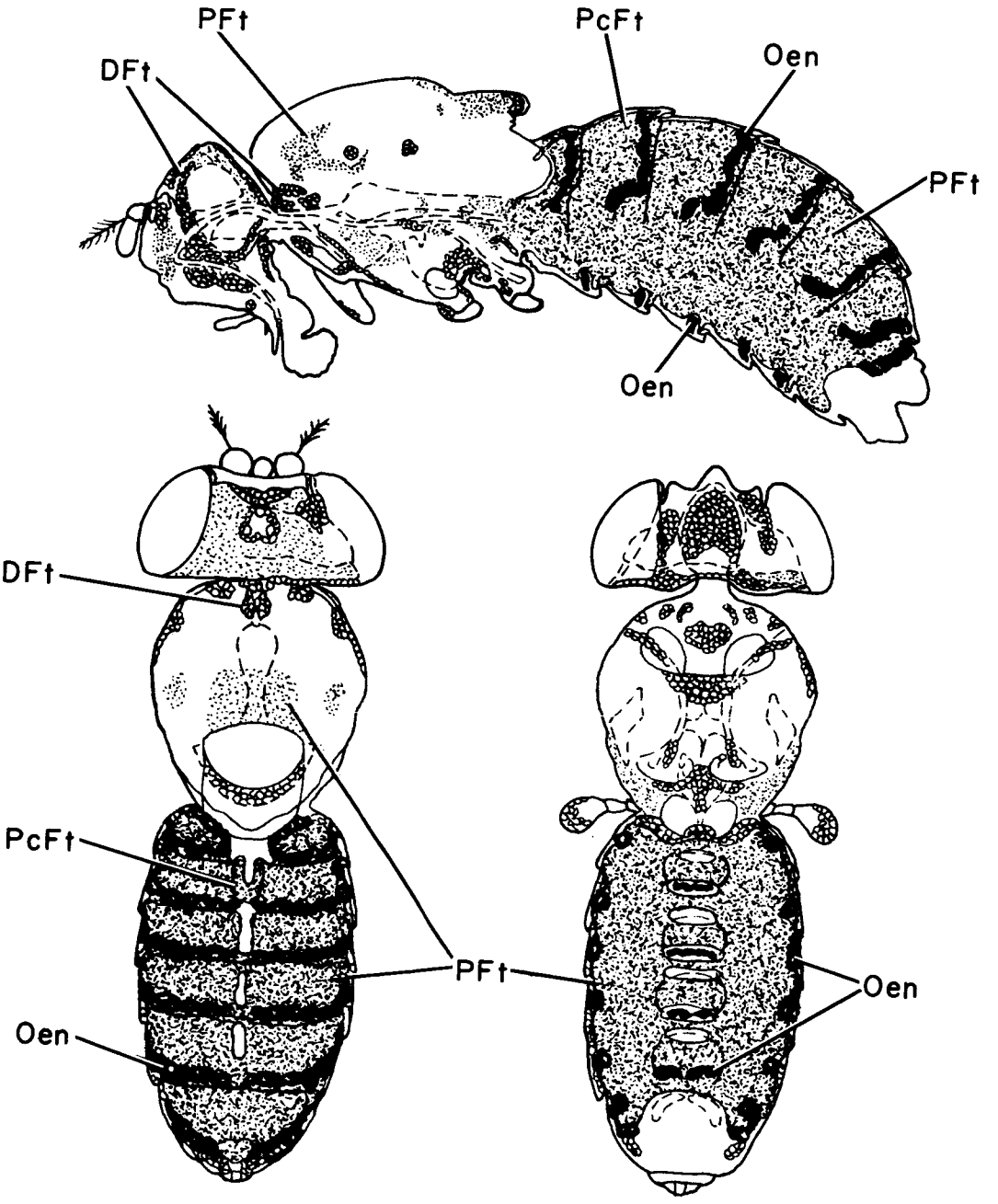

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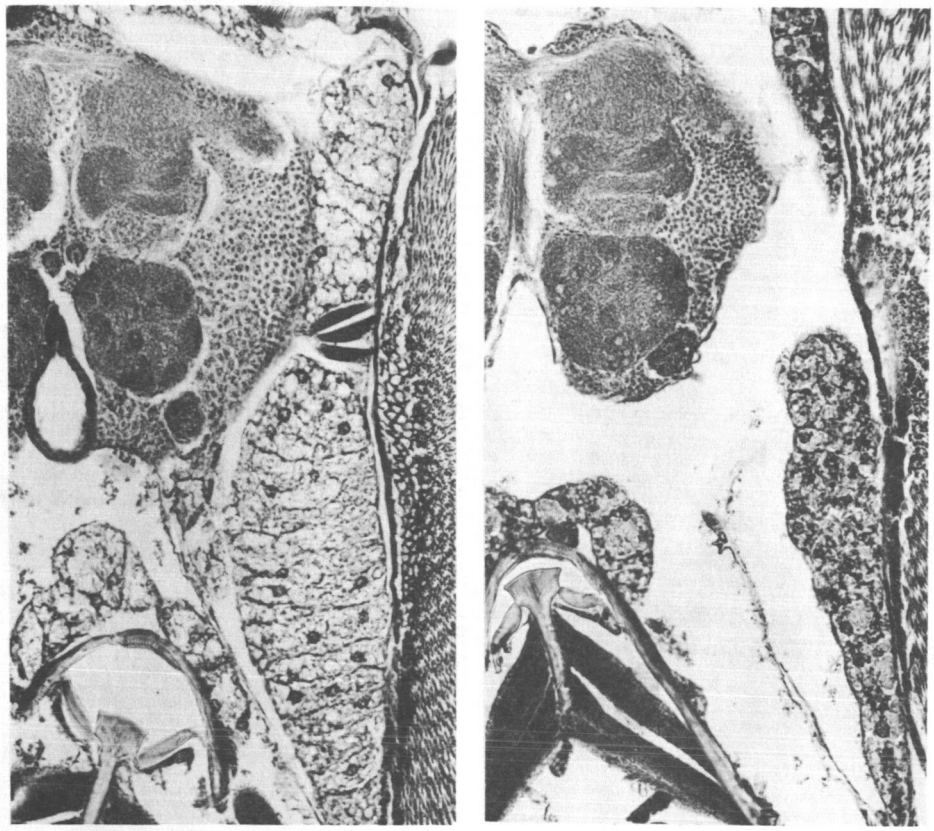

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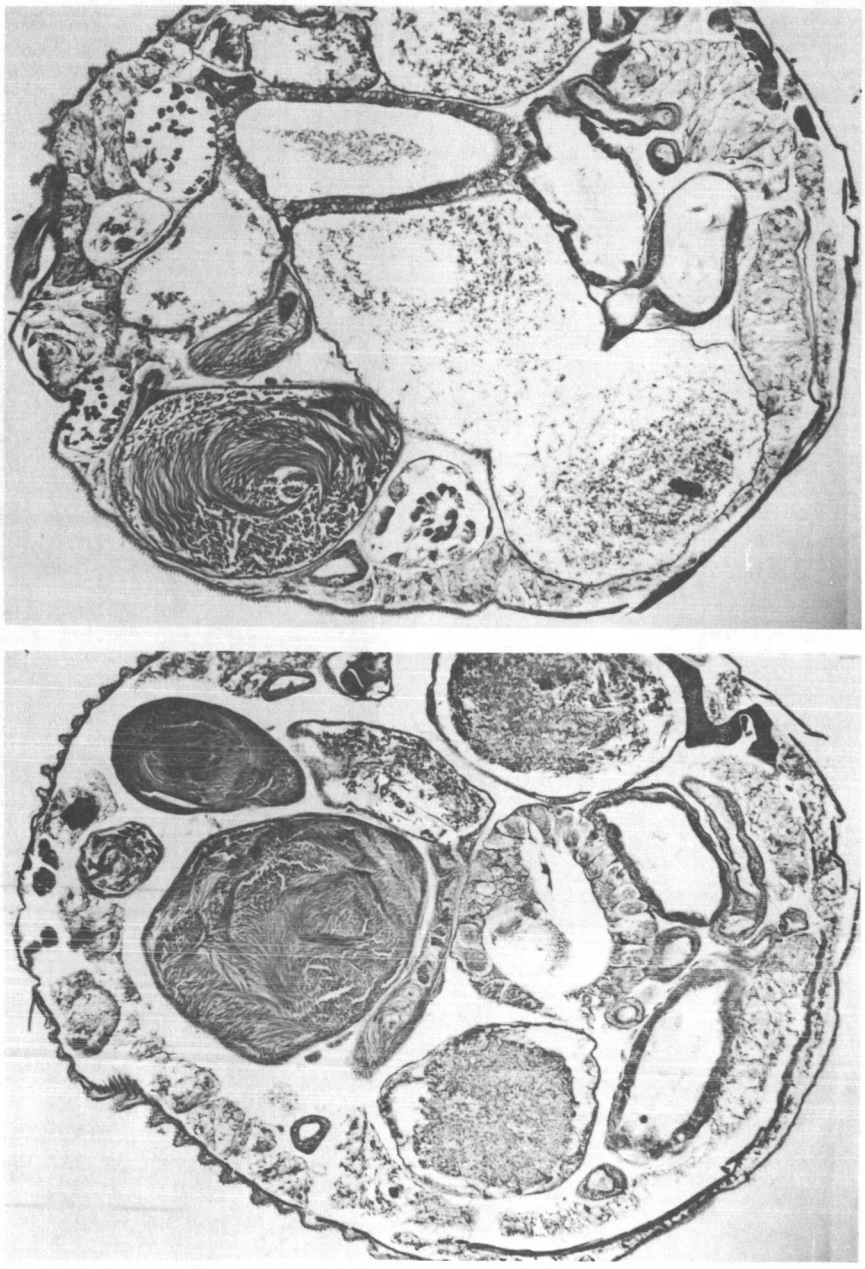

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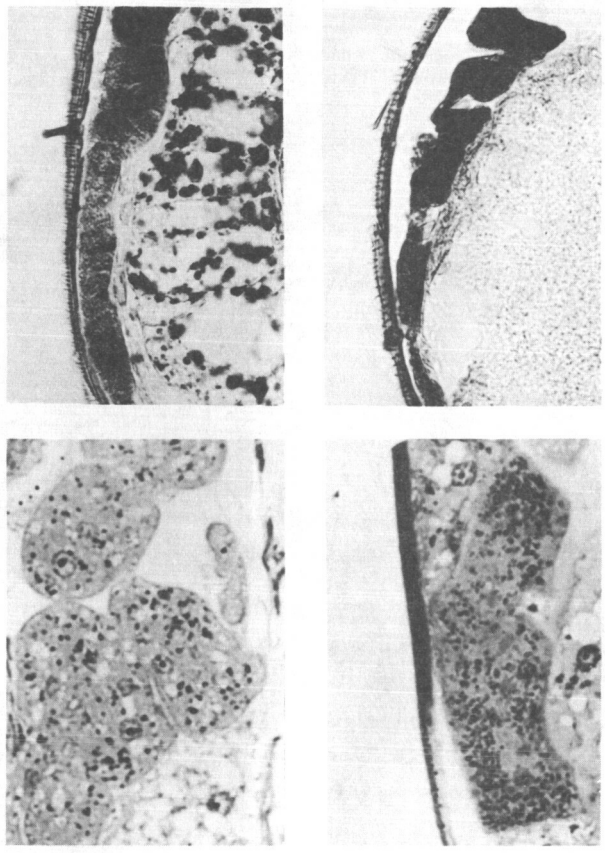

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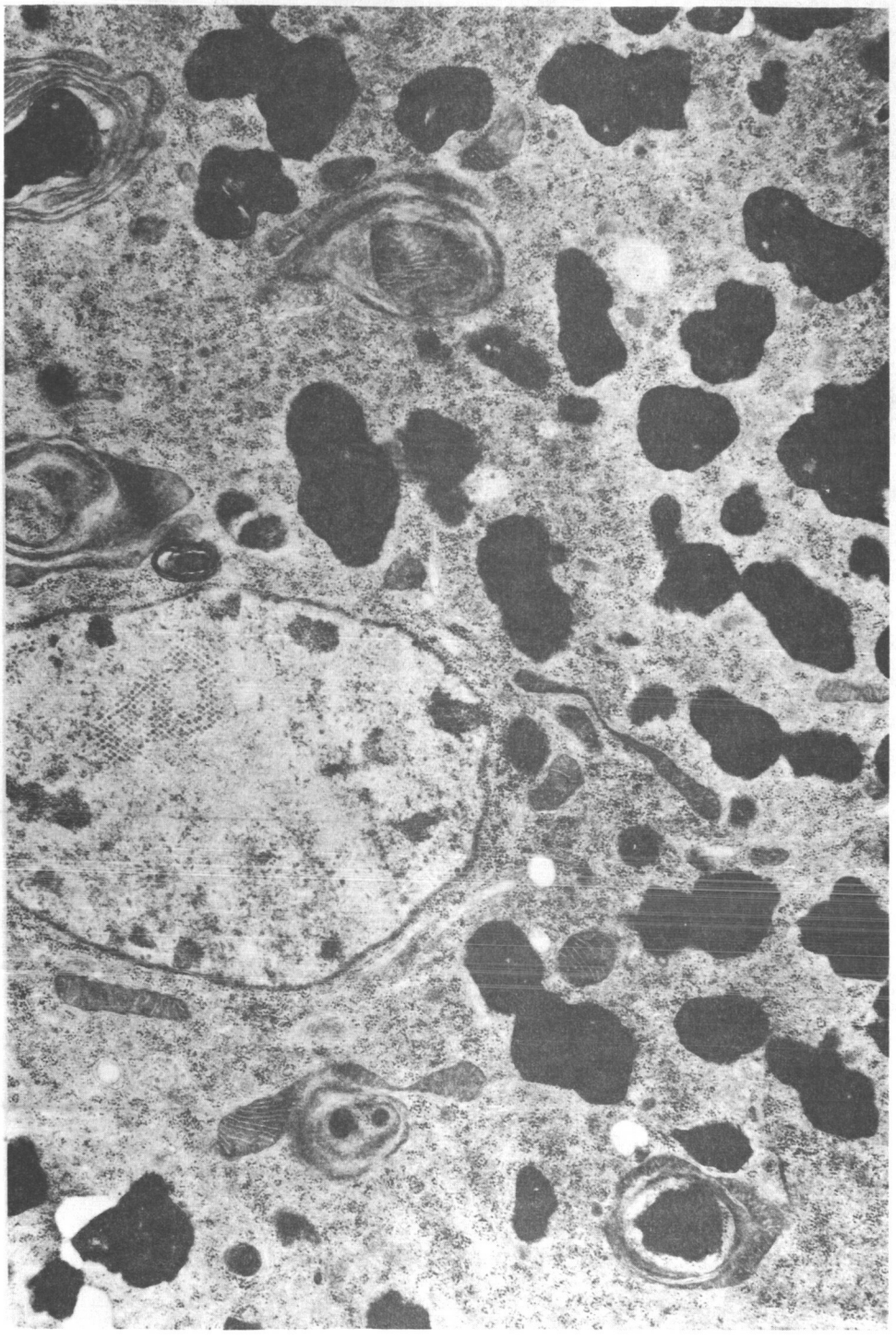

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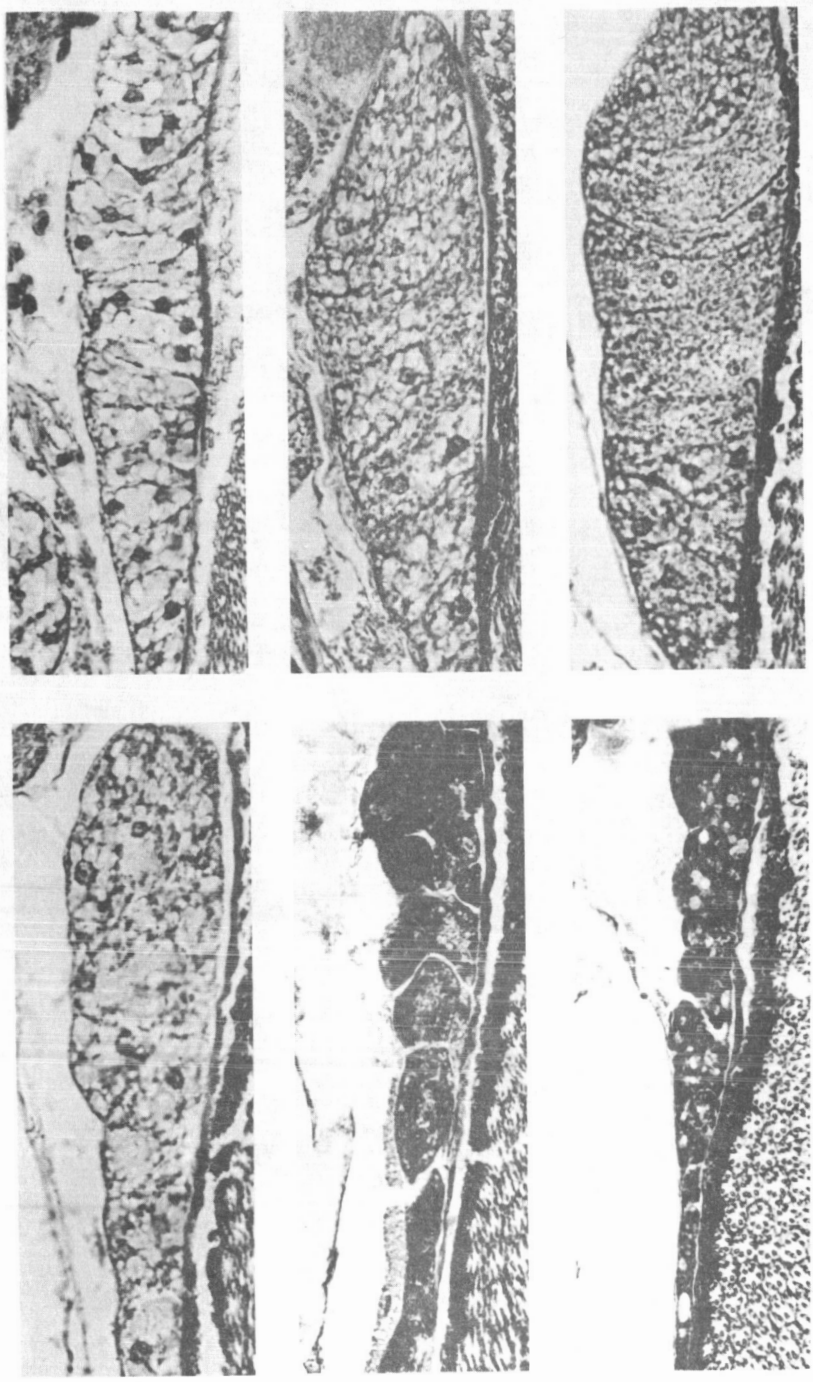

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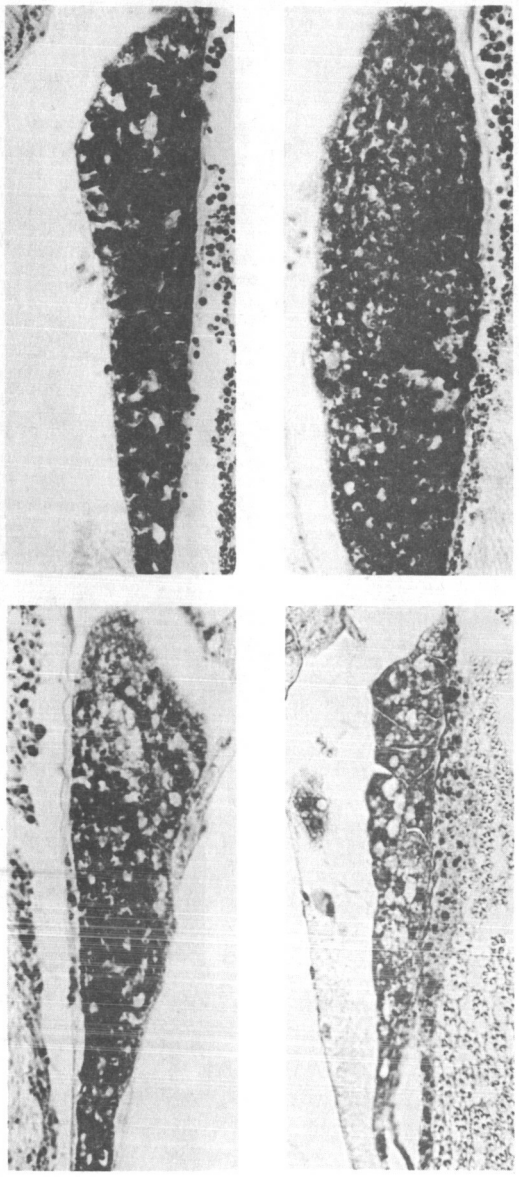

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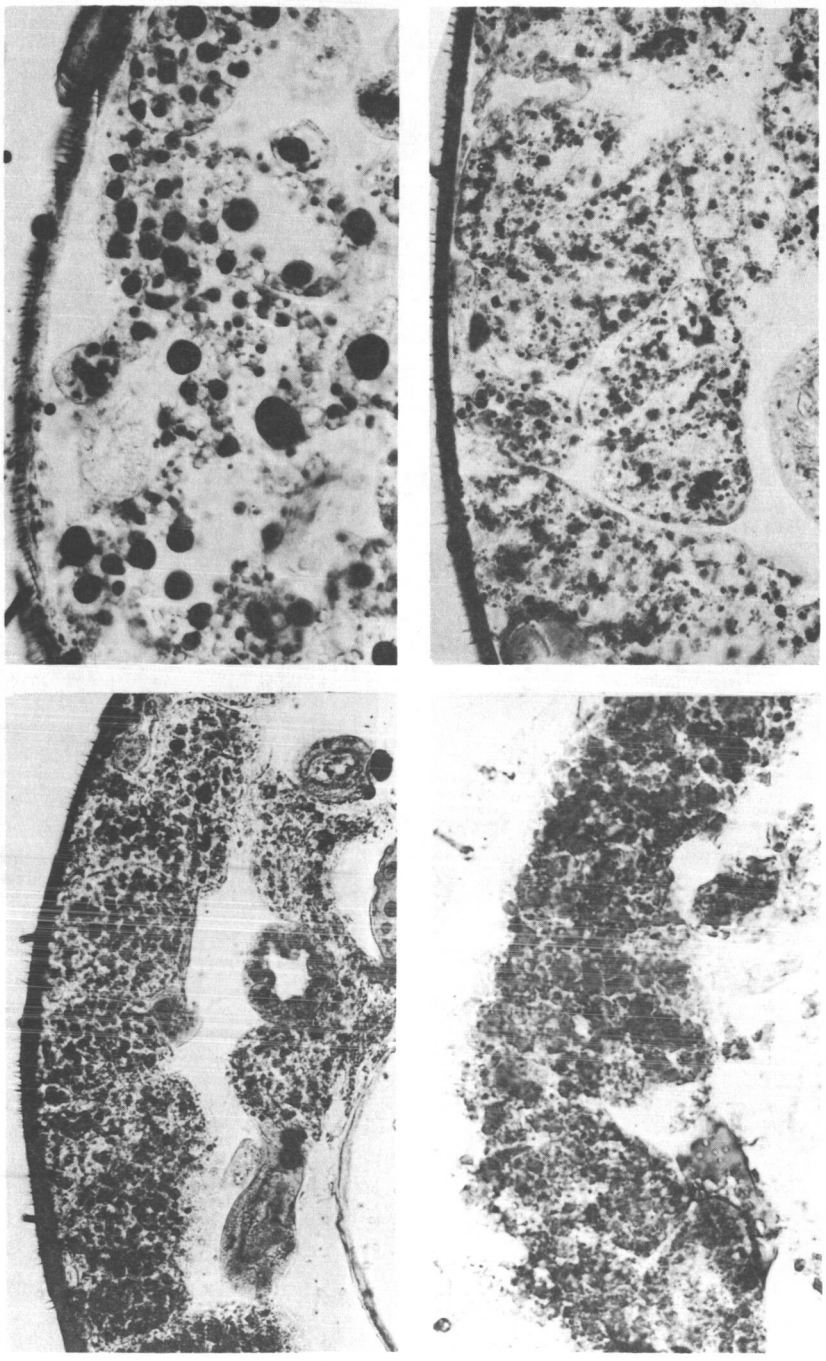

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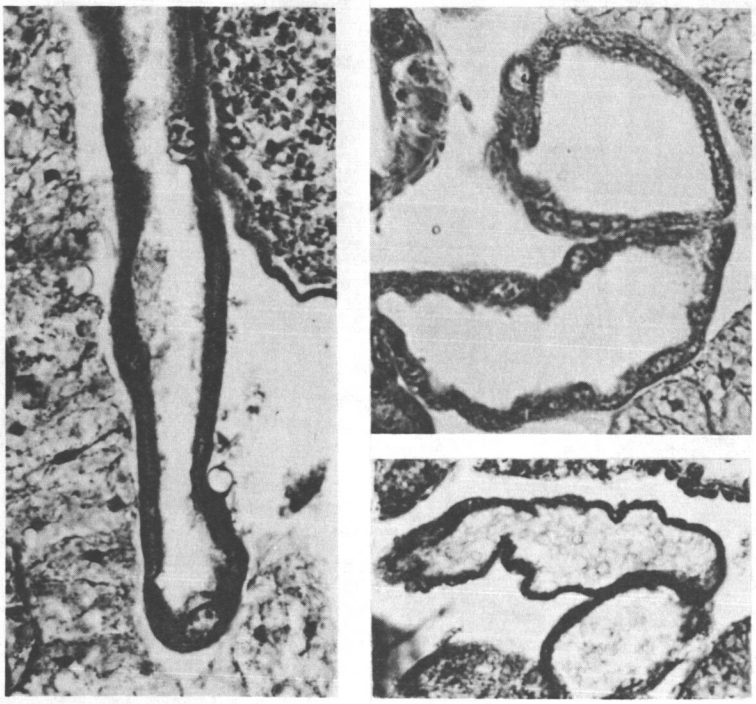

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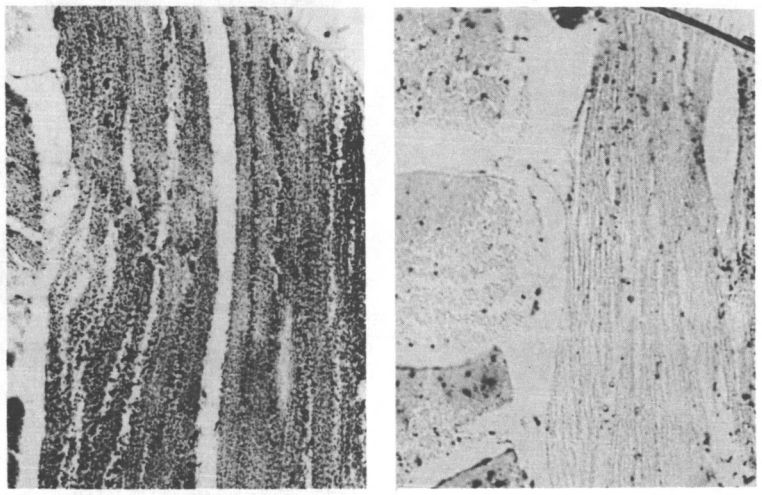

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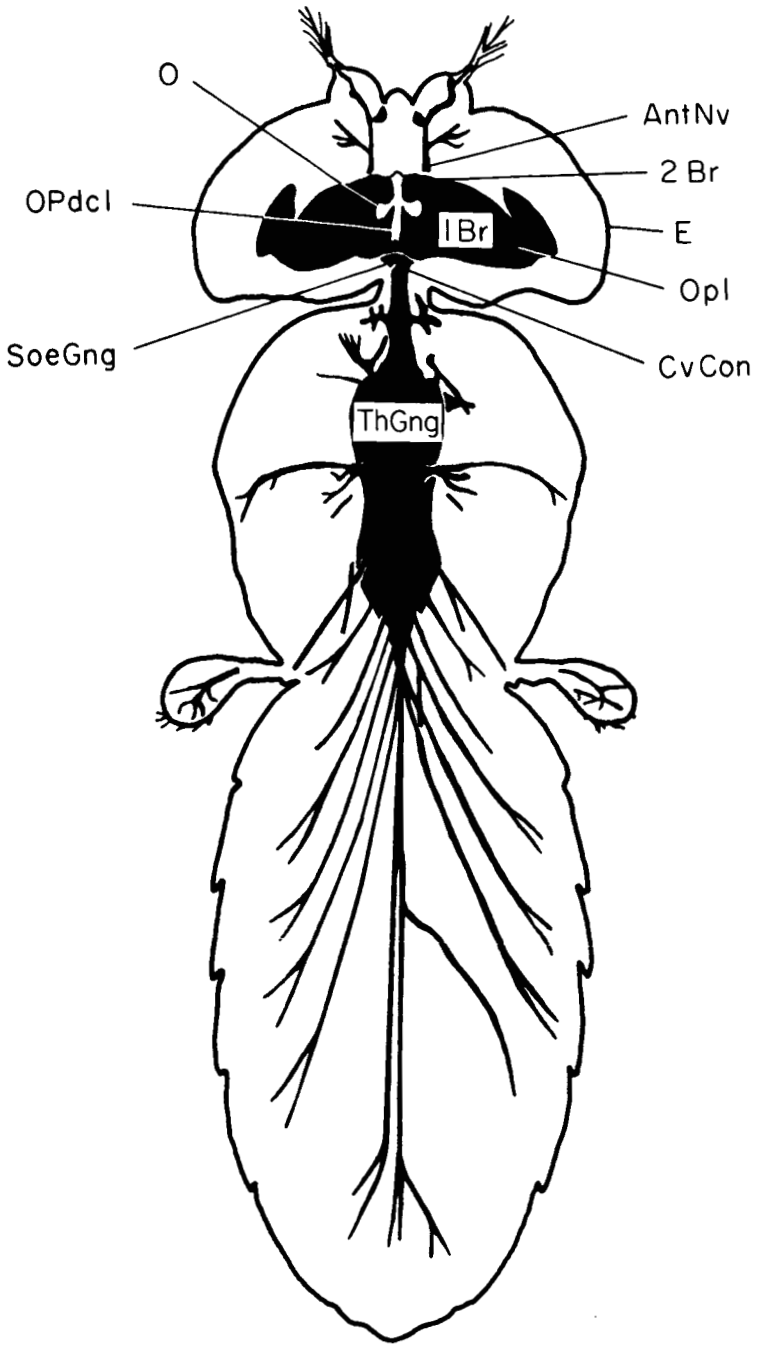

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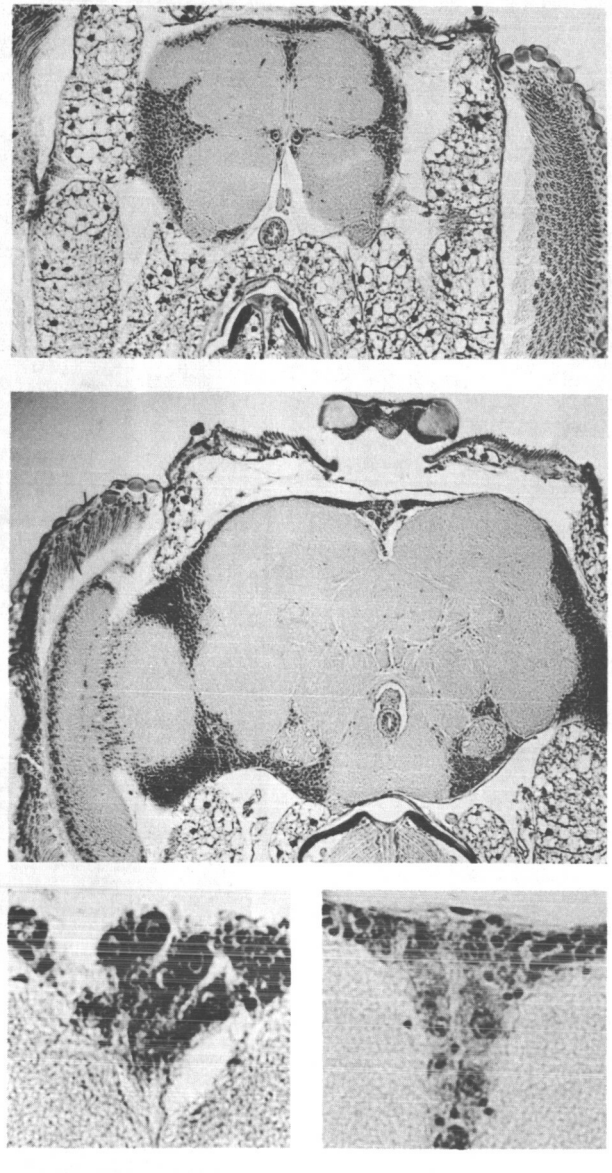

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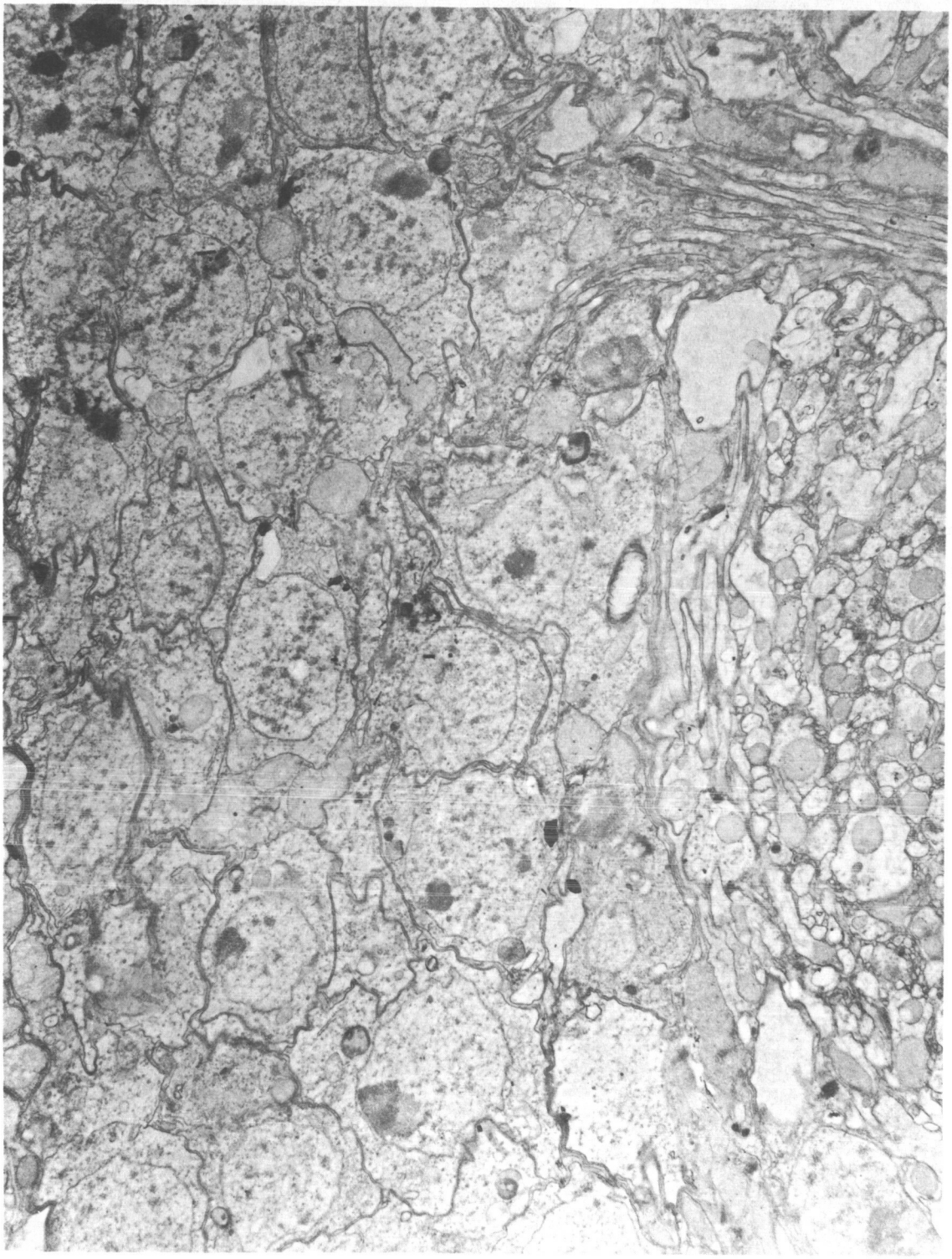

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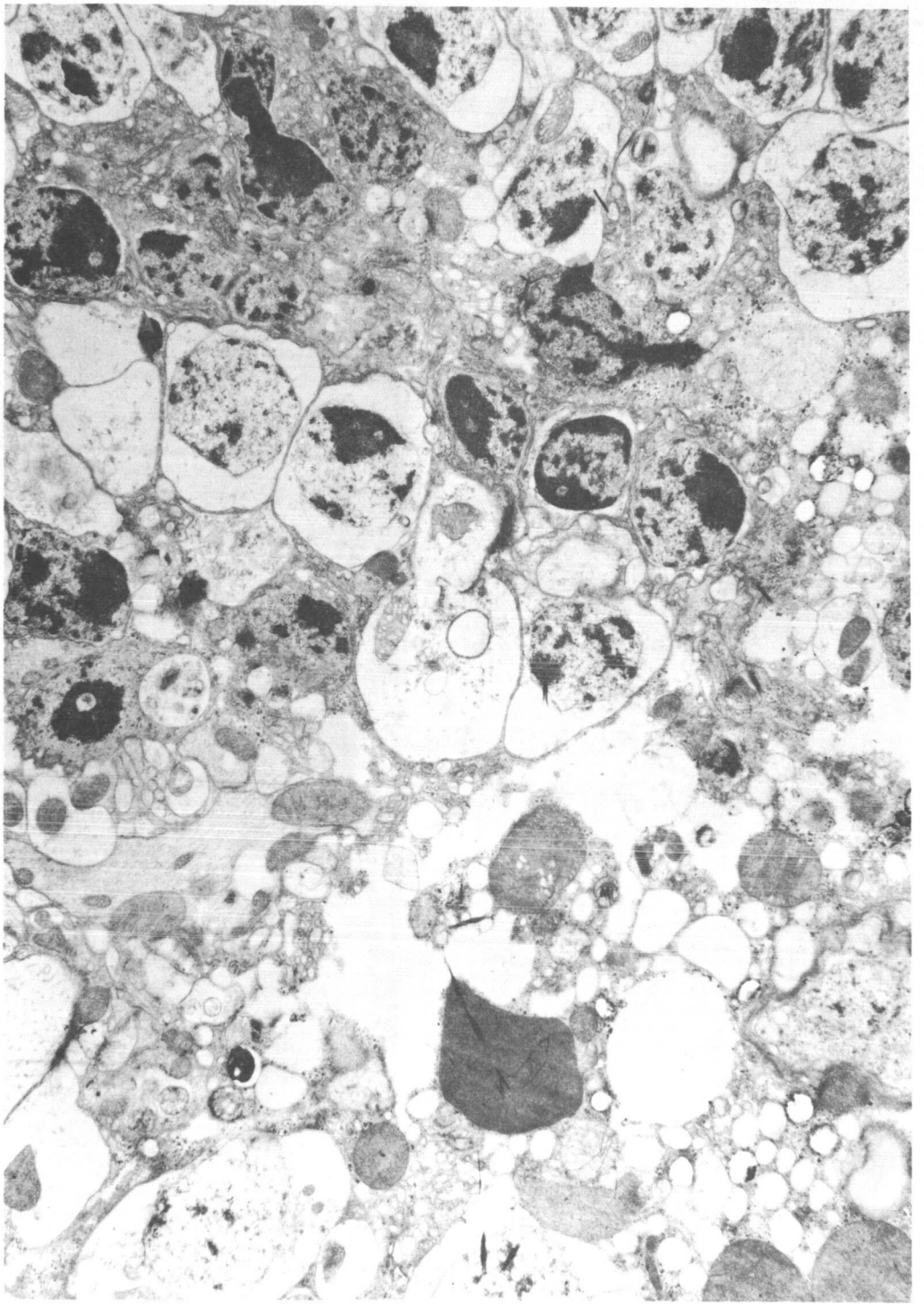

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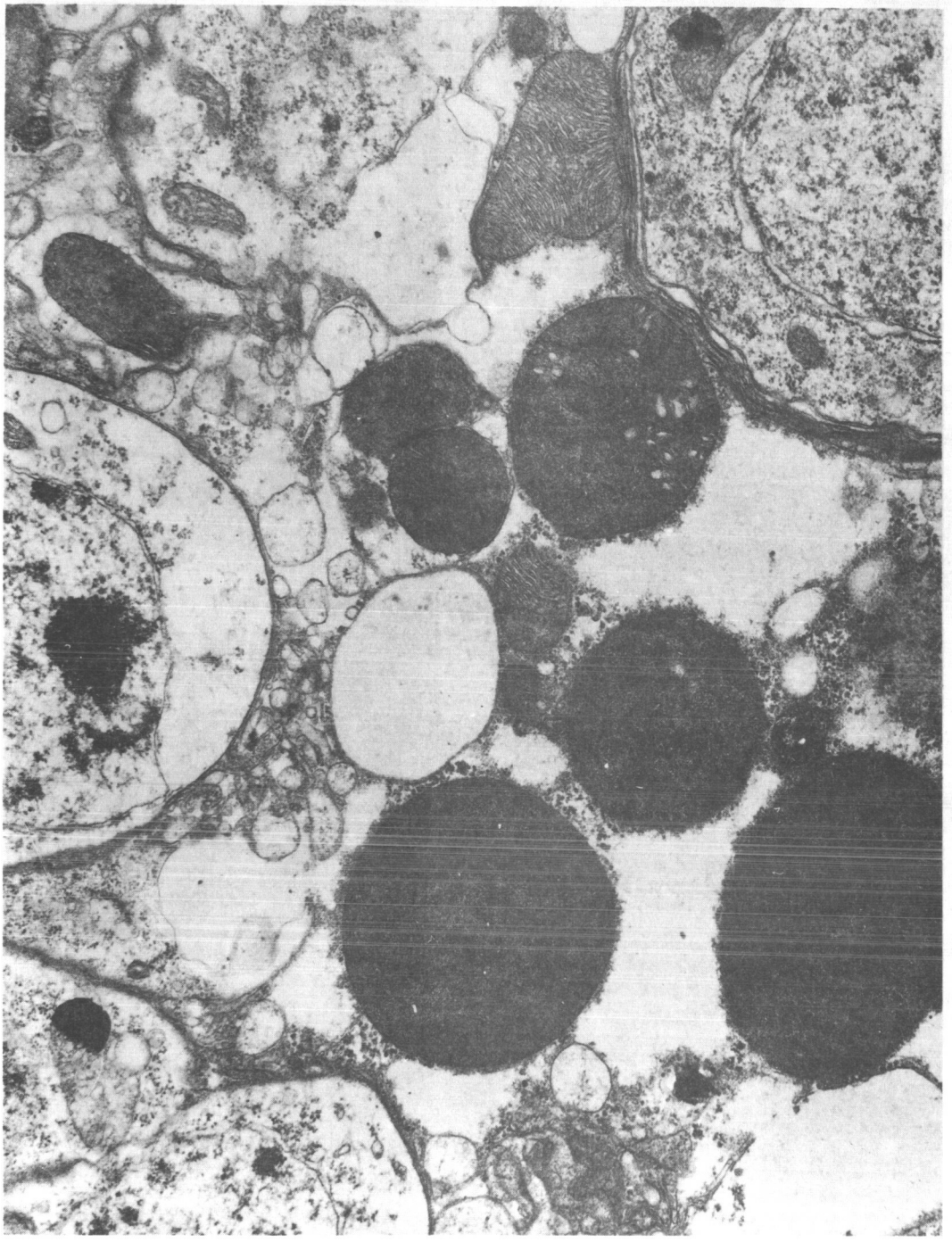

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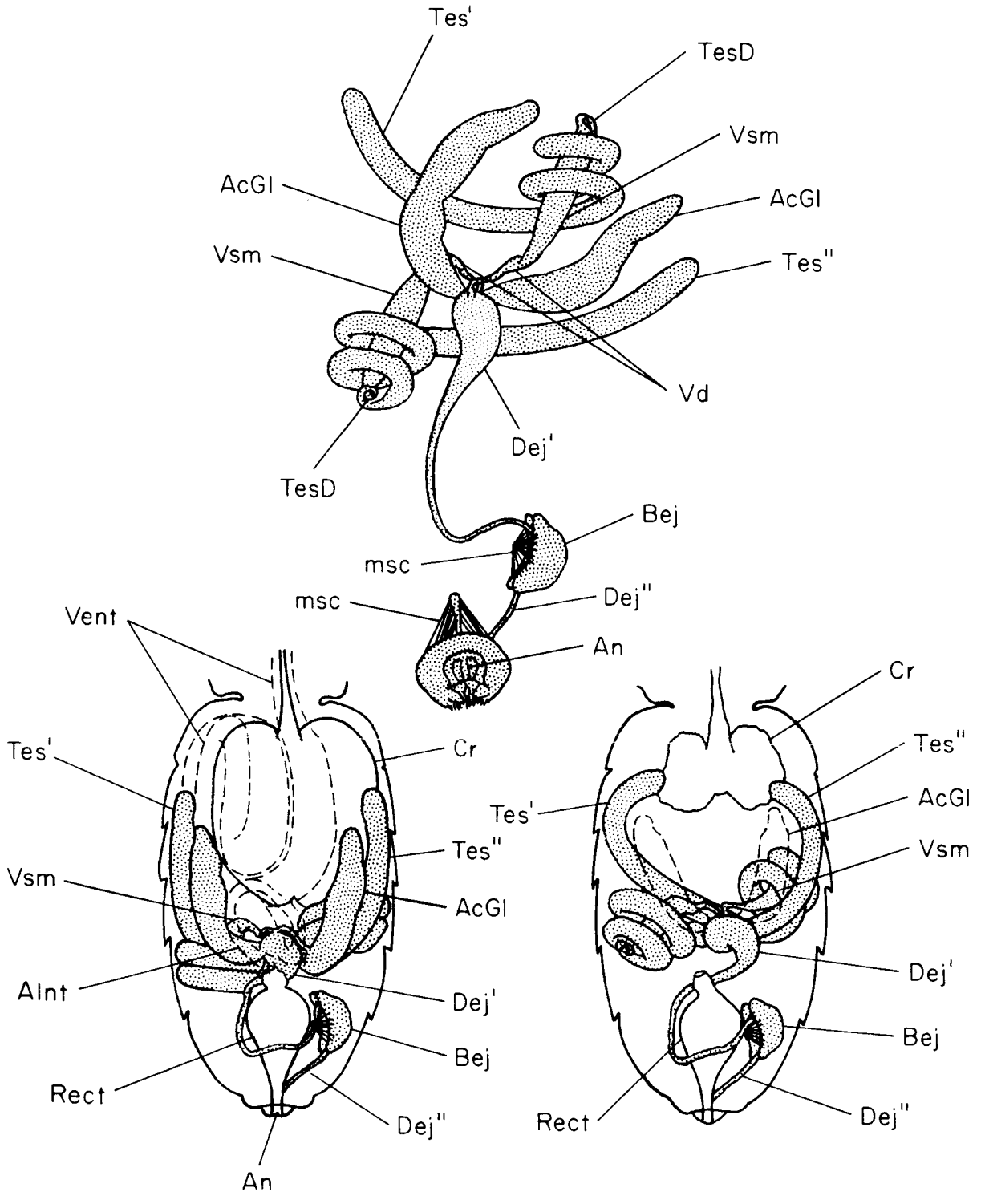

Figure 20.

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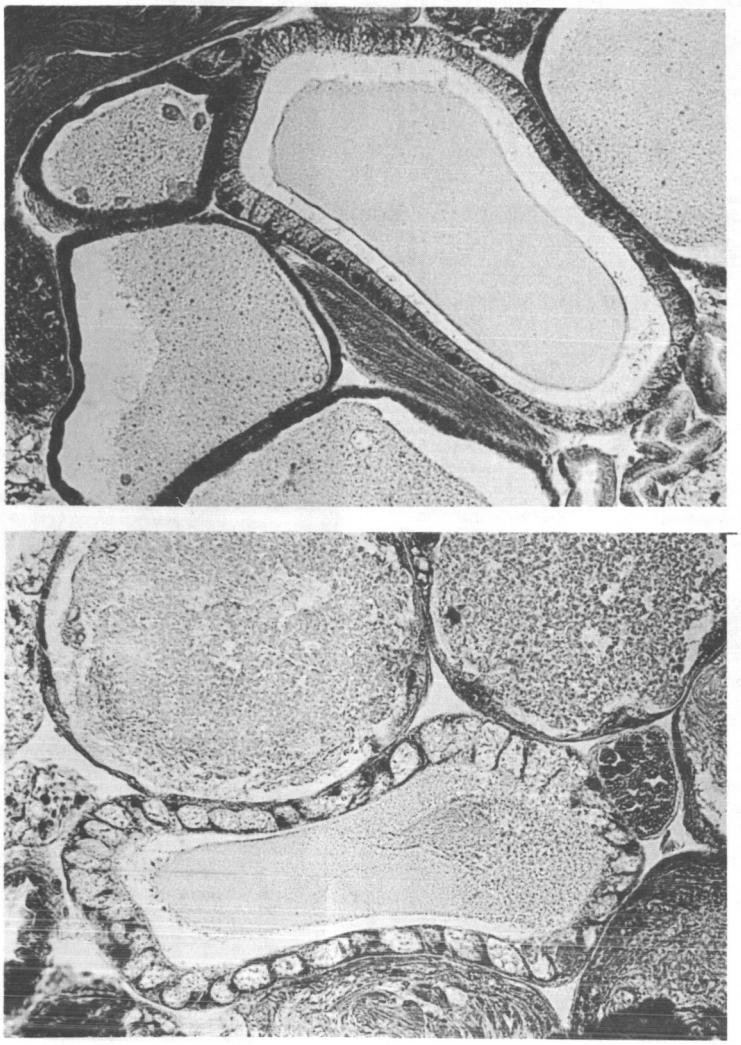

Figure 21.